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Growth, physiology, and leaf ion concentration responses to long-term flooding with fresh or saline water of *Populus euphratica*

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ABSTRACT

We aimed at evaluating morpho-physiological responses of *Populus euphratica* to flooding with fresh and saline water to assess its potential for conservation of lowland areas prone to suffer soil waterlogging. One-year-old cuttings were subjected to five treatments, including control, and flooding at increasing salinity concentrations (0, 50, 100, and 150 mM NaCl) for 90 days. Plant survival and growth, leaf gas exchange, leaf water potential, and concentration of Na^+, K^+, Ca^{2+}, Mg^{2+} in leaves were assessed. Flooding with saline water of 100 or 150 mM NaCl compromised plant survival. Plant death was preceded by strong reductions in photosynthesis, stomatal conductance and leaf water potential, increments in leaf Na^+ concentration, and restricted adventitious rooting. By contrast, flooding with fresh or slightly saline water (50 mM NaCl) did not endanger survival although it reduced final plant biomass (65–68%). Cuttings flooded with fresh or slightly saline water showed profuse development of adventitious roots and hypertrophied lenticels in stems as typical adaptive responses to hypoxia, and despite having smaller-sized leaves, these were able to continue photosynthesizing at levels of 40–50% of their controls even after 90 days of flooding. So, *P. euphratica* appears as a promising candidate species to be included in conservation programs for riparian areas experiencing long-term flooding with fresh or slightly saline water of up to 50 mM NaCl.

1. Introduction

Understanding the structural and functional responses of vegetation to combined flooding and salinity stress is essential to deal with increased storms and rising sea levels in future due to global warming (Martin et al., 2011). There is also an increasing international interest in conservation and protection of vegetation suffering from flooding (Fisher et al., 2011). Among abiotic stresses, flooding has been considered as one of the most important factors determining vegetation distribution (Dwire et al., 2004), mainly due to the decreased availability of oxygen in the soil (Armstrong, 1979; Li et al., 2011). Studies have shown that growth rate in a number of species in marshes decreases with long last flooding, although the responses are not always linear and may vary among species (Kirwan and Guntenspergen, 2012; Voss et al., 2013). Prolonged flooding leads to high physiological stress for vascular plants as root water uptake is reduced, impacting on leaf water potential and, consequently, on stomatal conductance reducing carbon assimilation (Colmer and Voesenek, 2009; Striker, 2012). Mineral nutrient concentration in leaves in response to flooding is expected to vary according to plant species tolerance and soil characteristics (Kozlowski, 1984; Pezeshki, 2001; see also reviews by Shabala, 2011 and Zeng et al., 2013, 2014 for detailed mechanisms of tolerance). Formation of adventitious roots of high porosity (i.e. aerenchyma content) helps to continue with water and nutrient uptake under oxygen-deficient conditions, thus aiding at alleviating the impact of flooding on shoot physiological activity (Striker et al., 2005; Di Bella et al., 2015). So, in this paper, we assessed a complete set of physiological variables like leaf water potential, stomatal conductance, photosynthesis, transpiration, and root adventitious formation to understand their relation with plant growth as affected by flooding with fresh and saline water.

Salinity affect negatively the growth of riparian plants, especially in species subjected to tides with fresh or saline water (Guo and Pennings, 2012). High salinity can reduce plant water potential, cause ion toxicity, and provoke reductions of nutrient uptake (Gorham et al., 1985). Given to the rising of sea level due to climate change, it is expected an increase of flooding events on saline areas where productivity of many marsh and swamp species may be reduced in the future (Parker et al., 2011). Stress caused by flooding and salinity promotes accelerated senescence of leaves through chlorophyll degradation along with stomatal closure, thereby reducing leaf photosynthetic functional capacity for carbon assimilation and plant growth (Baker, 2008). In addition to deprivation of oxygen, flooding with saltwater also might cause ion toxicity through the

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accumulation of Na^+ and Cl^- and reduction of K^+ in plant tissues (Munns and Tester, 2008). Some plant species growing under saline-flooded conditions store higher concentrations of salt ions than species growing in saline-dry conditions (Barrett-Lennard, 2002). For example, *Eucalyptus globulus* (Marcar et al., 1993) and *Casuarina obesa* (Carter et al., 2006) accumulate more salt in saline-flooded conditions compared to saline-dry conditions. Root functioning is impaired under hypoxia, and the membrane potential of roots is typically reduced to a range of −60 to – 80 mV (Zeng et al., 2014); this membrane depolarization provokes the activation of K^+-efflux channels determining that under hypoxia-saline conditions, roots are not able to retain enough K^+ (Shabala and Pottosin, 2014). In addition, the low energy (ATP) produced under limiting oxygen conditions might constrain the plant ability to exclude Na^+ by carriers (Colmer and Flowers, 2008), and it might potentially alter on other ions reaching the leaves as Ca^{2+} and Mg^{2+}. Therefore, in this paper, we measured ion concentration in leaves as well as leaf physiological behavior to understand the main constraints for plant growth in *Populus euphratica* when salts (NaCl) are present in the flood water.

Populus euphratica olive is among the species with unknown potential adaptability to saline-waterlogged environments. *P. euphratica* is a tree species growing naturally in riparian of arid and semi-arid areas in which seasonal floods and flooded soil provide favorable conditions for this tree species. The distribution range of this species extends from North Africa, across the Middle East and Central Asia to western China. *P. euphratica* has been reported as highly tolerant to dry and saline soils with pH up to 9.9 and a variety of textures including sand, loam, and clay (Viart, 1988). Besides its economic value as wood, *P. euphratica* is also used extensively to protect and to stabilize riparian zones, to preserve natural ecosystems, and to provide wildlife refuges (Daneshvar and Modir-Rahmati, 2006). Interesting studies have been carried out on the responses of *P. euphratica* species to salinity stress alone (Daneshvar and Modir-Rahmati, 2006; Han et al., 2013; Rajput et al., 2015) or to flooding with fresh water (Yu et al., 2015). Nevertheless, the responses to saline flooding of this species have not been assessed. In this study, we aim at investigating the morphological, physiological, and growth responses of *P. euphratica* to flooding stress with fresh as well as saline water to evaluate its potential for conservation and/or restoration of riparian areas prone to suffer from water excess.

2. Materials and methods

2.1. Plant species and experimental design

2.1.1. Plant growing conditions before treatments application

In the present study, *Populus euphratica* woody cuttings were prepared in fall (late February) from Alborz Research Center (35°48′N and 51°00′E) and planted in March in polyethylene pots (2 l) containing loam-sandy soil. Pots were transported in early May to a greenhouse of the Faculty of Natural Resources, located in Noor city, Iran (36°35′N and 52°02′E). Before transplanting, electrical conductivity (EC) of pots soils was measured in 3 replicates in laboratory, which was about 0.250 dS m^{-1}. Weeding was performed manually every 2 weeks. When reaching a height of 50–60 cm, cuttings were transplanted to larger pots (5 l) containing loam-sandy soils (without fertilizer) to prevent pot-size constraint for root growth. Pots were daily watered to field capacity until treatments application.

2.1.2. Treatments application

Previous to impose saline flooding treatments, a number of 1-year-old homogeneous cuttings with average diameter of 7.3 mm and average height of 63.3 cm were selected to provide 9 replicates per treatment. Afterwards, five treatments including control (non-flooding, non-saline), flooding with fresh water (0 mM NaCl), flood water with 50 mM NaCl, flood water with 100 mM NaCl, and flood water with 150 mM NaCl were imposed following a completely randomized design for 90 days (from mid-July to mid-October). Water level of flooding treatments was maintained at 4 cm above soil surface. To avoid a sudden osmotic shock to cuttings, the addition of NaCl to reach each salt concentration was gradual in three steps at days 1, 7, and 14 after flooding imposition (Pezeshki, 2001). Electrical conductivity (EC) of flood water and soil of pots was measured once a week in order to adjust salinity concentration of each treatment when needed (see Supplementary Material FigS1.). During the experiment, mean, minimum, and maximum temperatures in the greenhouse 20.2 °C and 36.2 °C (respectively), and humidity ranged between 21% and 74%.

2.2. Measurements

2.2.1. Survival and morphological parameters

Given the high stress exerted by flooding at increasing salinity concentration, which provoked the dead of individuals, survival of cuttings was recorded at the end of the experiment. Morphological parameters such as the diameter at 1 cm from the base and total height of cuttings were measured at the beginning and at the end of the experiment. Diameter was measured with digital caliper with a precision of 0.01 mm. In addition, the formation of adventitious roots and hypertrophy lenticels above the root shoot junction was weekly recorded.

2.2.2. Mean leaf area, SLA, and biomass

At the end of experiment, six leaves per seedling were taken from the uppermost part of the stem, weighed, and the leaf area was measured using a leaf area meter (Yang et al., 2007). Then, mean leaf area and specific leaf area (SLA, leaf area: leaf dry mass ratio) were assessed (Yin et al., 2009). Cuttings of each treatment were dissected into roots, stem, and leaves. Roots were classified in main (pre-existent) roots and adventitious (new) roots. All material was oven-dried at 70 °C for 48 h (Yin et al., 2009) and weighted.

2.2.3. Physiological parameters

Photosynthesis, transpiration rate, and stomatal conductance were measured from the beginning until the end of the experiment every 2 weeks. These physiological parameters were measured on sunny days using a portable infrared gas analyzer Model LCpro + (ADC BioScientific Ltd., Hertfordshire, UK) on fully expanded leaves located in the uppermost part of the stem (Gardiner and Krauss, 2001; Oguchi et al., 2006). Physiological parameters were measured in constant conditions of CO_2 (350 ppm), relative humidity (60–80%), and leaf temperature (25–30 °C) (Baltzer and Thomas, 2007).

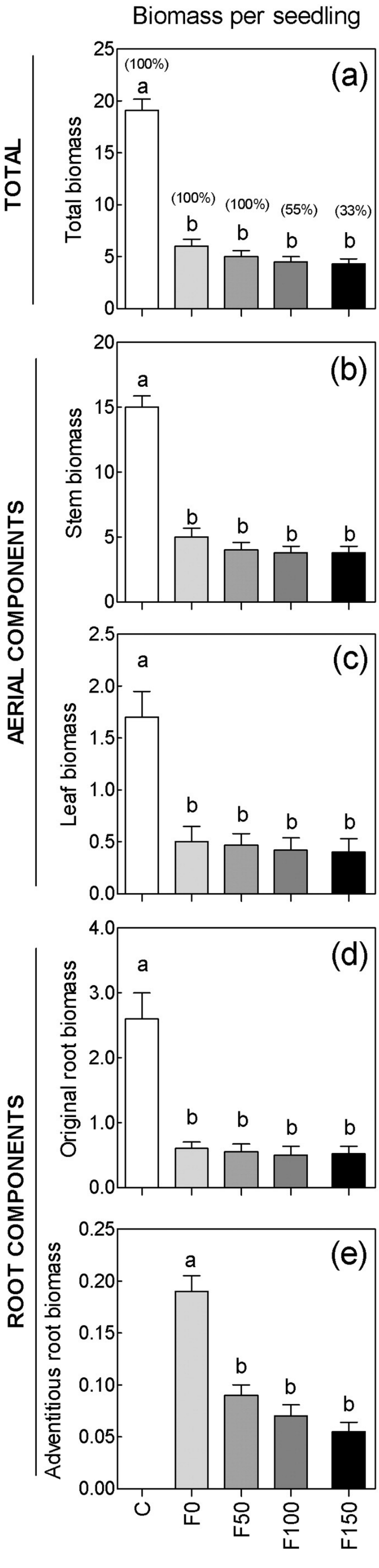
2.2.4. Leaf water potential and relative water content (RWC)

Leaf water potential (Ψ) was measured at the end of the experiment using a pressure chamber model Skye SKPM 1400 (Skye Instruments, Powys, UK) on similar leaves than those for carbon assimilation parameters. To measure the RWC of leaves, we used the methodology as in Martinez et al. (2007). Firstly, the uppermost younger leaves were removed and immediately weighed to obtain their fresh weight (WF). Secondly, those leaves were incubated in tightly closed tubes containing distilled water for 6 h to obtain their turgid weight (WT). Finally, the leaves oven dried at 70 °C by 48 h to obtain their dry weight (WD). So, the relative water content of leaves was obtained using Eq. (1):

$$RWC = \frac{WF\text{-}WD}{WT-WD} \times 100 \tag{1}$$

2.2.5. Leaf chlorophyll

To measure leaf chlorophyll, samples of 0.1 g of fully expanded leaves (upper positions in the stem) were transferred to test tubes covered with aluminum foil. Then, 3 ml of dimethylformamide was added to each sample; test tubes were sealed with teflon to avoid dimethylformamide leakage. Samples were incubated at 4 °C for 3 days (Riethmuller-Haage



et al., 2006) and their absorbance at wavelengths of 647 and 664.5 nm was recorded by using a spectrophotometer (FCC Compliance (Epoch), Biotek Instrument, USA). Chlorophyll concentration was calculated using the equations proposed by Inskeep and Bloom (1985) and expressed as milligram per gram of leaf fresh weight.

2.2.6. Leaf ion concentrations

Leaf samples from upper portions of cuttings were taken and the concentration of Na^+, K^+, Ca^{2+}, and Mg^{2+} in leaves was measured following Riberio et al. (2002). To do this, samples were dried in an oven at 68 °C for 48 h and ground to fine powder (*ca.* 1 g per sample) and mixed with 10 ml of concentrated nitric acid (HNO_3). After 12 h of incubation in the hood, samples were maintained at 80 °C for 2 h. After cooling, 3 ml of concentrated perchloric acid ($HClO_4$) was added to the samples and incubated at 160 °C for 5 h. Afterwards, samples were cooled and filtered with filter paper topped up with distilled water to a volume of 25 ml. Concentrations of abovementioned ions were determined by AAS (atomic absorption spectrometry; Philips, PU9400X, England).

2.3. Data analysis

Physiological parameters such as photosynthesis, stomatal conductance, and transpiration were analyzed using repeated-measures ANOVA with Duncan test for mean comparison among treatments. Sphericity assumption of covariance matrix was tested using Mauchly's test (Von Ende, 1993). One-way ANOVA was used to determine the level of significance for all other parameters with Duncan tests as *a posteriori* comparison of means. Data were checked to satisfy ANOVA assumptions of normality of data and homogeneity of variances using Kolmogorov–Smirnov and Levene's tests, respectively. Data were analyzed using SPSS.17 statistical software.

2.4. Results

2.4.1. Survival, biomass, and morphological responses to flooding with fresh or saline water

Flooding with saline water at concentrations higher than 50 mM affected not only plant biomass accumulation but also plant survival. Only half of plants (55%) survived 90-d of stress when subjected to flooding with 100 mM NaCl, and 3 out of 9 plants (*ca.* 33%) survived when saline water contained 150 mM NaCl. Importantly, flooding with fresh or slightly saline water (50 mM NaCl) did not provoke plant death. Flooding caused a reduction in total biomass of *ca.* 68% irrespective of salt concentration (Fig. 1a) where, as expected, roots were more affected (79–87% of reduction; Fig. 1d) than stem and leaves (biomass reduced in a range of 67–78% (stem) and 70–82% (leaf) with respect to controls; Fig. 1b,c). It was notorious that adventitious root biomass was 3 times higher in cuttings subjected to flooding with fresh water with respect to those that experienced a saline flooding at 150 mM NaCl (Figs. 1e; 5ab).

Seedling morphology was affected by flooding. Plant height increase was similarly reduced (55–58%) by flooding with either fresh or saline water (Fig. 2a). Stem diameter was also reduced by flooding (35% with respect to controls) while addition of salt provoked further decreases in this parameter (compare control, flooding with fresh water *vs.* flooding with saline water in Fig. 2b). In the same line, mean leaf size was also reduced by 44–67% as a consequence of flood-induced hypoxia irrespective of salt addition treatment (Fig. 2c). The reduction

Fig. 1. Total biomass (a), leaf biomass (b), stem biomass (c), original root biomass (d), and adventitious root biomass (e) of 1-year-old *Populus euphratica* cuttings under different flooding conditions. C: control, F0: flooding with fresh water, F50: 50 mM NaCl in the flood water, F100: 100 mM NaCl in the flood water, and F150: 150 mM NaCl in the flood water. Flooding lasted 90 days. Different letters indicate significant differences between treatments at $P < 0.05$ based on Duncan tests. Numbers between brackets in (a) indicate the percentage of surviving cuttings. Values are means ± standard error of 9 replicates.

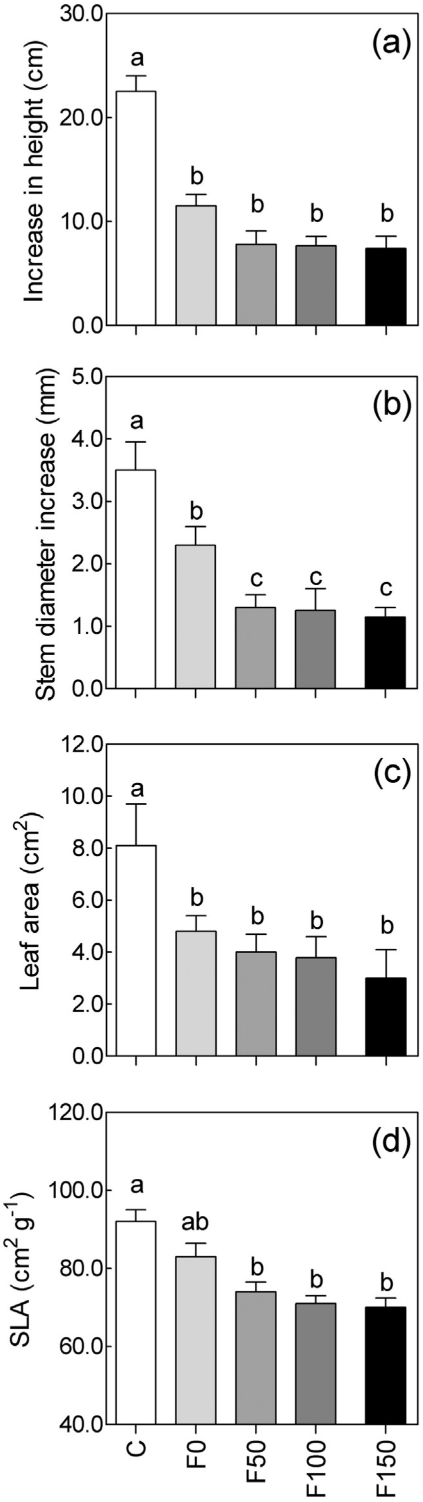


Fig. 2. Plant height increase (i.e. final height − initial height) (a), diameter increase (b), leaf area (c), and SLA (specific leaf area) (d) of 1-year-old *Populus euphratica* cuttings under different flooding conditions. C: control, F0: flooding with fresh water, F50: 50 mM NaCl in the flood water, F100: 100 mM NaCl in the flood water, and F150: 150 mM NaCl in the flood water. Flooding lasted 90 days. Different letters indicate significant differences between treatments at $P < 0.05$ based on Duncan tests. Values are means ± standard error of 9 replicates.

in leaf size was slightly higher than the reduction in leaf mass under saline flooding (compare Fig. 2c with Fig. 1b) with respect to control cuttings, and, as a consequence, the specific leaf area (SLA; cm^2 g^{-1}) was slightly reduced (Fig. 2d).

2.4.2. Physiological responses as affected by flooding with fresh or saline water

Flooding reduced the levels of photosynthesis (A), stomatal conductance (g_s), and leaf transpiration (E) of *P. euphratica* cuttings progressively along the experiment with respect to control plants (rmANOVA; treatment × time effect: $P < 0.05$). The magnitude of the reductions in A, g_s, and E depended on the salt concentration of the flood water. Flooding with saline water with increasing concentrations caused further reductions than those provoked by flooding with fresh water (Fig. 3a,b,c). At the end of experiment (day 90), the values for photosynthesis under non-saline flooding, flooding with 100 mM NaCl, and flooding with 150 mM NaCl dropped to values of 61%, 74%, 83% of their respective controls (Fig. 3a). The reductions in E paralleled the progressive closing of stomata along flooding time (compare Figs. 3b and c).

Leaf water potential of *P. euphratica* cuttings decreased by 28% under non-saline flooding with respect to controls, and it was further reduced when flood water contained 150 mM NaCL (Fig. 4a). The relative water content in leaves (RWC) varied with the treatments matching the results obtained for leaf water potential (Fig. 4b). Thus, more stressful conditions, namely, flooding at increasing salt concentration, provoked progressive reductions in leaf RWC (Fig. 4b). In the same line, chlorophyll concentration was reduced 17% by flooding with fresh water, and it was further reduced at increasing salt concentration to reach less than 50% of the values of controls (9.5 ± 0.14 mg g^{-1} of DW) when flood water contained 150 mM NaCl (see Fig. S2).

2.4.3. Ion concentrations in leaf tissues

Flooding at increasing saline concentrations provoked progressive accumulation of Na^+ in leaves, suggesting that this species is not able to regulate well Na^+ exclusion in roots and/or its transport to the leaves (Table 1). Concentrations of K^+ and Ca^{2+} in leaves were reduced by *ca.* 42% and 32.7% (respectively) when cuttings were subjected to saline flooding of either NaCl concentration. Leaf Mg^{2+} was reduced (24%) by saline flooding with 50 mM NaCl but further reduced (42.1–53.7%) when concentration of salt increased in the flood water to 150 mM of NaCl. The K^+:Na^+ ratio decreased 38% as a result of flooding with fresh water mainly because a slight trend to increase leaf Na^+ concentration (although no significant) and a maintenance of leaf K^+ concentration (see Table 1). As expected, at high salt concentrations (100 and 150 mM NaCl) in the flood water, the K^+:Na^+ ratio in leaves was strongly reduced due to accumulation and reduction of Na^+ and K^+ in leaves, respectively, again suggesting a low ability of this species to deal with flooding containing high levels of NaCl.

2.5. Discussion

This study showed that *Populus euphratica* looks promising for stabilization and/or restoration of (i) marginally saline riparian areas prone to suffer from prolonged water excess in the soil (electrical conductivity up to 4 dS/m; Barrett-Lennard, 2002; Brady and Weil, 2002) and (ii) coastal areas receiving flooding with slightly saline water (up to 50 mM NaCl). In this experiment, flooding with fresh water and flooding with 50 mM NaCl revealed some signs of stress on the plants, but there were no marked signs of accelerated senescence, necrosis, and/or leaf falling (as occurred under higher saline concentration in the flood water; see Fig. 5c and Kozlowski, 1997). It seems that the morphological and physiological adjustments (e.g., formation of adventitious roots, maintenance of photosynthesis, and stomatal aperture) displayed by this species to face a prolonged flooding period of 90 days (with fresh water or slightly saline) were the main reasons explaining the survival of the cuttings. On other hand, it was also clear that when salt concentration in the flood water surpassed 50 mM NaCl, this species was not able to adjust its physiology and its survival was compromised (Fig. 5c). At such high salt concentration in the flood water, *P. euphratica* could not avoid the excessive leaf Na^+ accumulation (*cf.* Di Bella et al., 2014, 2015) nor develop a comparable

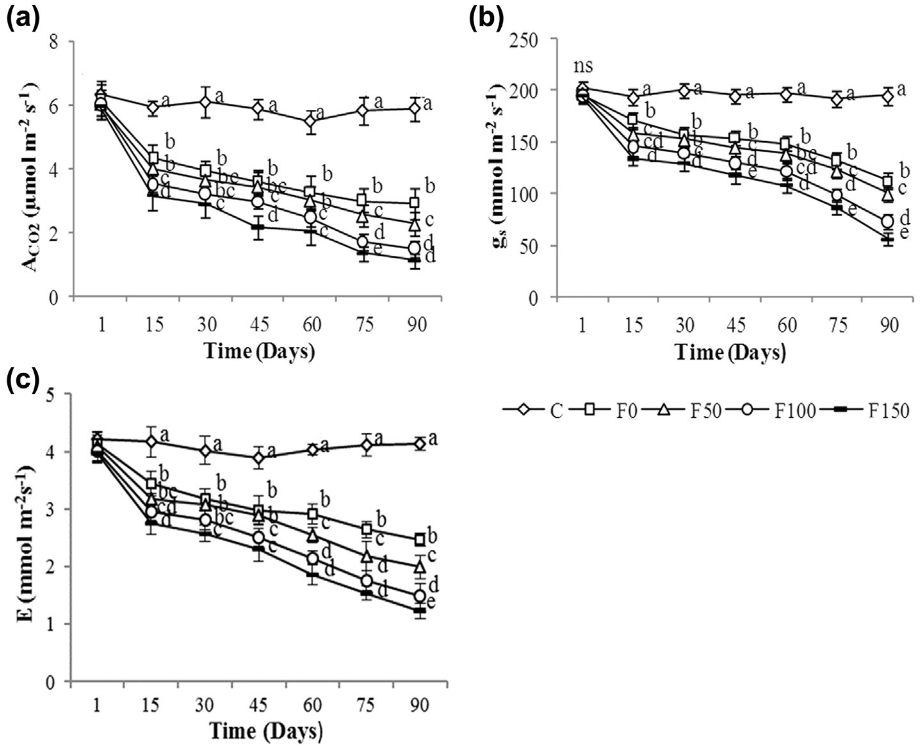


Fig. 3. Photosynthesis (A_{CO2}; a), stomatal conductance (g_s; b), and transpiration (E; c) of 1-year-old *Populus euphratica* cuttings under different flooding conditions. C: control, F0: flooding with fresh water, F50: 50 mM NaCl in the flood water, F100: 100 mM NaCl in the flood water, and F150: 150 mM NaCl in the flood water. Flooding lasted 90 days. Different letters indicate significant differences between treatments at $P < 0.05$ based on Duncan tests within each date of measurement. Values are means ± standard error of 9 replicates.

adventitious rooting than when flooded in fresh water. Those responses, in concert with leaf nutrient imbalances (less Ca^{2+} and Mg^{2+}) and the potentially toxic effects of Na^{+} (and Cl^{-}) could be the causes by which cuttings perished (Homaee, 2002). It is important to note that assessment of target areas to introduce this species should pay special attention to soil salinity rather than floods *per se*. Also, complementary studies at field conditions are necessary to confirm the results of the present experiment under natural conditions where this species will perform if it is introduced.

2.5.1. Morphology and growth

Combined flooding–salinity stress had a significant effect on the height and diameter growth of *P. euphratica* cuttings. Decreased growth of *P. euphratica* cuttings may be due to some constraint in root aeration and reduction in absorption of water and nutritional elements as well as, to a lesser extent, denitrification processes (Kozlowski and Pallardy, 2002). In this study, diameter and height growths in flooding–salinity conditions declined more compared to flooding with fresh water, which is similar to the results of Krauss et al. (1999) on *Taxodium distichum* seedlings and Conner et al. (1997) on *Fraxinus pennsylvanica* and *Sapium sebiferum* seedlings in flooding with salinity levels of 0, 2, and 10 g/l. Also, Carter et al. (2006) showed that the relative height growth rate of flooded seedlings of *Casuarina obesa* exposed to salinity of 0.01, 200, and 400 mM NaCl decreased at increasing salinity.

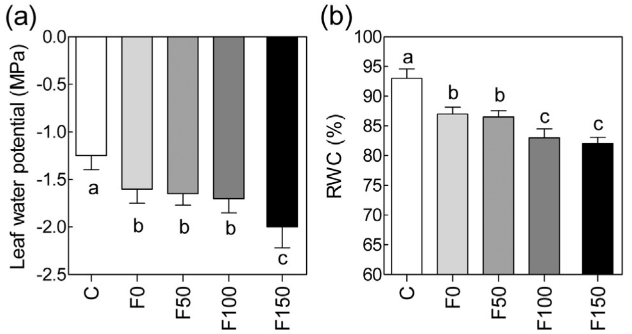


Fig. 4. Leaf water potential (a) and RWC (relative water content in leaves) (b) of one-year-old *Populus euphratica* cuttings under different flooding conditions. C: control, F0: flooding with fresh water, F50: 50 mM NaCl in the flood water, F100: 100 mM NaCl in the flood water and F150: 150 mM NaCl in the flood water. Flooding lasted 90 days. Different letters indicates significant differences between treatments at $P < 0.05$ based on Duncan tests. Values are means ± standard error of 9 replicates.

Increased salinity level under flooding conditions caused decrease in biomass of main pre-existent roots, leaves, and stem biomass of *P. euphratica* cuttings (Fig. 1). Similar results under combined flooding–salinity stress have been reported on *Pinus taeda* (Pezeshki, 1992), *Sporobolus virginicus* (Naidoo and Mundree, 1993), *Taxodium distichum* (Allen et al., 1994), and *Jatropha curcas* (Gimeno et al., 2012). Generally, flooded soils influence negatively on plant growth through production of ethanol and toxic metabolites in hypoxic tissues, lower production of ATP due to anaerobic metabolism, and decreased photosynthesis at plant basis (Fig. 3a) associated to reduced chlorophyll and smaller-sized leaves (Fig. 2c; Fig. S2; Bernardino and Carvalho, 2000; Striker et al., 2005, 2011). In this study, about 1 week after flooding, adventitious roots appeared over the stems (above the root shoot junction) of all *P. euphratica* cuttings immersed in water (Fig. 5ab; Parad et al., 2015). Adventitious rooting is an important well-knowledge adaptive response to mitigate hypoxia by flooding (Colmer and Voesenek, 2009; Striker, 2012). In our experiment, biomass of these special roots was progressively reduced with increasing concentration of NaCl in the flood water, similar to that reported by McCarron et al. (1998) on *Cephalanthus occidentalis* and by Carter et al. (2006) on flooded seedlings of *Casuarina obesa* and *Melaleuca cuticularis*. Song et al. (2011) also showed that flooding with high concentrations of NaCl (i.e. 200, 600 mM) impaired the formation of adventitious roots on the *Suaeda salsa* (a true halophyte species) where the number and length of adventitious roots were reduced at increasing salinity. In addition, we observed hypertrophied lenticels appearing on stems of flooded *P. euphratica* cuttings after 2 weeks of flooding

Table 1
Concentration of Na^+, K^+, Ca^{2+}, Mg^{2+}, and K^+:Na^+ ratio in leaves of *Populus euphratica* cuttings under different flooding conditions. C: control, F0: flooding with fresh water, F50: 50 mM NaCl in the flood water, F100: 100 mM NaCl in the flood water, and F150: 150 mM NaCl in the flood water. Flooding lasted 90 days. Different letters indicate significant differences between treatments at $P < 0.05$ based on Duncan tests.

	C	F0	F50	F100	F150
Leaf Na^+ (g. kg^{-1})	0.45 ± 0.05^{d}	0.71 ± 0.06^{d}	15.85 ± 0.06^{c}	25.55 ± 0.9^{b}	28.62 ± 0.1^{a}
Leaf K^+ (g. kg^{-1})	2.35 ± 0.04^{a}	2.3 ± 0.03^{a}	1.44 ± 0.05^{b}	1.38 ± 0.02^{bc}	1.28 ± 0.35^{c}
Leaf Ca^{2+} (g. kg^{-1})	0.58 ± 0.035^{a}	0.54 ± 0.017^{a}	0.42 ± 0.025^{b}	0.39 ± 0.02^{b}	0.36 ± 0.018^{b}
Leaf Mg^{2+} (g. kg^{-1})	2.14 ± 0.05^{a}	2.03 ± 0.08^{a}	1.64 ± 0.07^{b}	1.24 ± 0.06^{c}	0.99 ± 0.03^{d}
K^+:Na^+ ratio	5.34 ± 0.51^{a}	3.26 ± 0.3^{b}	0.091 ± 0.004^{c}	0.054 ± 0.002^{c}	0.045 ± 0.002^{c}

(Fig. 5a; Parad et al., 2013), similar to that informed by Yu et al. (2015) for this species. It is argued that lenticels facilitate gaseous exchanges between plant tissues and the atmosphere, especially useful for oxygen diffusion to the plant and the release of potentially toxic compounds such as acetaldehyde, ethanol, and ethylene (Kozlowski, 1997; Glenz et al., 2006).

2.5.2. Gas exchange under flooding conditions with fresh or saline water

We proved that A, g_s, and E in *P. euphratica* cuttings decreased after 15 days' exposure to either flooding with fresh or saline water but in a different magnitude (Fig. 3). Although the decrease of these physiological parameters continued until the end of the experiment (day 90), cuttings flooded with fresh water had 2-fold higher values of A, g_s, and E than those flooded with water containing 150 mM NaCl. Reduced photosynthesis rate may be related to stomatal factors (stomatal closure) (Syvertsen et al., 1983; Striker et al., 2005) and non-stomatal factors, including changes in carboxylation efficiency, photochemical efficiency of photosystem II, chlorophyll concentration, and activity of photosynthetic enzymes (Yordanova and Popova, 2007). In the case of *P. euphratica*, our results suggest that both types of factors accounted for the drop in photosynthesis as stomatal closure was evident as well as chlorophyll reduction. However, we think that A reduction was explained in a greater extent by stomatal conductance as this variable perfectly paralleled the pattern of responses in photosynthesis while chlorophyll only dropped 17% in flooded cuttings with fresh water compared to controls. In addition, stomatal closure can be related to hydraulic (i.e. water potential) and non-hydraulic (i.e. hormonal) responses (Else et al., 1996; Naumann et al., 2008). In this study, the reduction of stomatal conductance appears to be related with progressively poor water status in leaves as reflected by the lower leaf water potential and RWC at increasing stressful conditions from flooding with fresh water to flooding with saline water (Fig. 4). These results are comparable with those of Ober et al. (2005) in sugar beet and Garcia-Sanchez et al. (2007) in citrus where RWC in leaves decreased with decreasing leaf water potential under flooding (and also drought stress).

The high magnitude in the reduction of A, g_s, and E in plants flooded with saline water may be also due to toxic effect of Cl^- and or Na^+ ions (Naumann et al., 2007). Here, stomatal closure can be related to further decrease of hydraulic conductivity (Kozlowski and Pallardy, 2002) of functional-impaired roots given the constrained development of

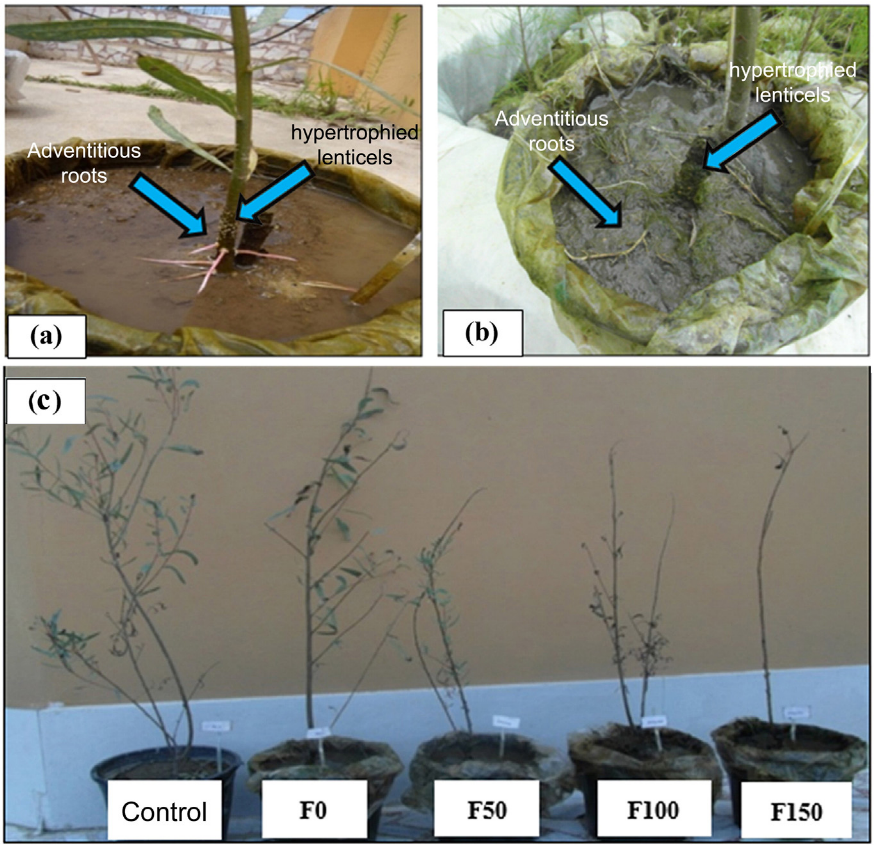


Fig. 5. Adventitious roots and hypertrophied lenticels of 1-year-old *Populus euphratica* cuttings subjected to flooding with fresh water by 14 days (a) and 90 days (b). In (c), it is shown representative pictures of 1-year-old *Populus euphratica* cuttings after 90-day growing at different flooding conditions. Control, F0: flooding with fresh water, F50: 50 mM NaCl in the flood water, F100: 100 mM NaCl in the flood water, and F150: 150 mM NaCl in the flood water.

adventitious roots when salt was added to the flood water (Fig. 1e). So, a reduced water uptake might explain the further reduction of RWC and leaf water potential registered in cuttings flooded with increasingly salty water. Such a loss of turgor of leaf tissues might in turn account for the high degree of stomata closure as a mechanism to prevent leaf dehydration by reducing leaf transpiration (Striker et al., 2005). In addition, the reduced transpiration under flooding with saline water could be attributed to the entering of Cl^- and Na^+ ions into the transpiration flow and therefore the damage to the transpirating leaf cells (Munns et al., 2005; Glenz et al., 2006). This last process might be expected to occur due to the high Na^+ concentration in leaf tissues in our experiment on seedling flooded with increasing NaCl concentration (Table 1). Here, the addition of salt in the flood water provoked a high decrease in chlorophyll concentration (50% of controls) suggesting that also non-stomatal factors were involved in the high reductions of A in seedling flooded with salty water (Fig. 3a).

2.5.3. Leaf concentration of Na^+, K^+, Ca^{2+}, and Mg^{2+} under flooding with fresh or saline water

In this research, the concentrations of calcium, magnesium, and potassium decreased while concentration of sodium increased at increasing salinity in the flood water in line with the results of Pezeshki et al. (1999) on *Quercus nuttallii* and *Q. falcata* seedlings and Duan et al. (2007) on *Suaeda salsa* seedlings. A potential explanation is that the lack of oxygen in the soil plus the excessive amount of Na^+ in the flood water (i.e. saline flooding) affected the energy production in root cells, therefore the selective incorporation and retention of K^+ and exclusion of Na^+ did not operate normally (Barrett-Lennard and Shabala, 2013). As a result, under saline flooding, K^+ uptake and transport to the leaves diminished, and thereby their concentration in leaves dropped to values of 54% with respect to controls at the highest salt concentration in the flood water (Table 1). Importantly, K^+ is not only an essential macronutrient in plants with widely known role in regulating stomatal aperture/closure (Mengel and Arneke, 1982; Benlloch-Gonzalez et al., 2008), but it can also act as a signaling molecule (Anschütz et al., 2014). So, the poor survival rate of cuttings exposed to high levels of salinity/hypoxia might be the result of root cells undergoing programmed cell death (PCD) due to increased endonuclease and protease activities following reduction in cytosolic K^+ (Shabala and Pottosin, 2014).

The massive accumulation of Na^+ in leaves of cuttings flooded with saline water suggests that the mechanisms for root exclusion, accumulation in stem, and/or diminished Na^+ xylem transport were not operative to prevent this toxic ion on reaching the leaves (Munns et al., 2005). The high concentration of Na^+ in leaves was probably the cause of an accelerated leaf senescence and abscission observed at the high salt concentration treatments (see Fig. 5c; Barrett-Lennard, 2003; Di Bella et al., 2014, 2015), and it would be, at least partially, responsible of inducing tissue toxicity and, ultimately, provoking plant death—in contrast to flooding with fresh water that did not affect plant survival. It was interesting to observe also that flooding with saline water derived in reductions in Ca^{2+} and Mg^{2+} concentration in leaves (Table 1). Nevertheless, it is still unclear if NaCl have induced (or not) Ca^{2+} or Mg^{2+} deficiencies in *P. euphratica* as the threshold of minimum concentration for these nutrients in leaves are not known for this (or a comparable) species. An interesting future approach to test this idea would be to provide plants with supplemental Ca^{2+} (and/or Mg^{2+}) and to register the responses in terms of survival and growth (see Gratten and Grieve, 1994).

3. Conclusions

Populus euphratica displayed important morpho-physiological adjustments to survive up to 3 months of flooding with fresh and saline water up to 50 mM NaCl, namely, maintenance of positive carbon fixation and development of adventitious roots and hypertrophied lenticels in stems. So, in the context of global warming effects leading to a rising sea level, this species can be considered suitable for further field studies confirming its potential for conservation and/or restoration of lowland and riparian areas experiencing long-term flooding events with fresh and/or or slightly saline water of up to 50 mM NaCl.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.sajb.2016.11.004.

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